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Research Article
Effects of Edible Insects on the Mycelium Formation of *Cordyceps militaris*

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Abstract
Background and Objective: *Cordyceps militaris* is a potential edible medicinal mushroom which containing various biological activity such as anti-inflammatory, anti-ageing, anti-protozoal and anti-microbial. The compositions of *C. militaris* media were composed of carbon source, nitrogen source and other additives. This research aimed to evaluate the effect of edible insects on the *C. militaris* mycelium formation. **Materials and Methods:** Seven edible insects including *Bombyx mori* L., *Samia ricini* D., *Acheta domesticus* L., *Gryllus bimaculatus* De Geer, *Tenebrio molitor* L., *Rhynchophorus ferrugineus* and *Lethocerus indicus* were used as nitrogen source supplemented in Potato Dextrose Agar (PDA) and the mycelium formation of each edible insects at day 7, 14 and 21 were recorded. **Results:** The results of nitrogen source from a boiled edible insect at day 21 indicated that the highest colony diameter at 88.00 mm was obtained when cultured with PDA+*B. mori* L. The results of nitrogen source from a dried edible insect at day 21 presented that the highest diameter at 84.33 mm was obtained from cultured using PDA+*A. domesticus* L. **Conclusion:** The suitable boiled and dried edible insects for the supplement in PDA were boiled *B. mori* L. and dried *A. domesticus* L. This is the first report about PDA supplemented with edible insects that can be increased the *C. militaris* mycelium formation which the initial stage that important for *C. militaris* cultivation.

Key words: *Cordyceps militaris* mycelium formation, nitrogen source, edible insects, *Bombyx mori* L., *Acheta domesticus* L., potato dextrose agar, anti-inflammatory, water agar

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cordyceps militaris is a valuable medicinal mushroom belonging to the family Cordycipitaceae (Ascomycota)[1] and a species of *Cordyceps* genus[2]. *Cordyceps militaris* is a parasitic fungus which their spore can enter into the pupa and larva of Lepidoptera insects and kill the host by feeding[2,3]. *Cordyceps militaris* produces an important various bioactive compound, such as polysaccharides, adenosine, cordycepin, mannitol, Superoxide Dismutase (SOD), carotenoids some polysaccharides[4]. Cordycepin is a structurally similar to adenosine, except that lack of the 3'-hydroxyl group and not the 3' position of the ribose part cordycepin[2,5]. Cordycepin is one substance the have many reports about their biological and pharmacological activities including immunological stimulating[6], anti-virus, antitumor, anticancer activities, cholesterol and triglycerides reduction[7],antioxidant and antiaging activities[8], improve lung and kidney function[9], anti-inflammatory[10] and hypotensive effects[11].

Cordyceps militaris mycelium is an important initial part of mushroom cultivation. The *C. militaris* mycelium growth depends on several factors such as carbon source nitrogen source, vitamin, temperature, pH and some environmental factors. Different media have been cultured for *C. militaris* mycelium such as Water Agar (WA), Potato Carrot Agar (PCA), Malt Agar (MA), Malt-Yeast Agar (MYA), Yeast-extract Malt-extract Peptone Dextrose agar (YMA), Sabouraud Maltose agar plus Yeast Extract (SMAY), Sabouraud Dextrose agar plus Yeast Extract (SDAY) and Potato-Dextrose Agar (PDA), etc.[12].

Nitrogen source is one of the important factors for *C. militaris* mycelium growth. Two types of nitrogen sources have been used including inorganic nitrogen and organic nitrogen sources[12]. Insects are one the organic sources that contain approximately 10% nitrogen by weight[13]. Bombay locust, scarab beetle, house cricket and silkworm were high protein-containing edible insects ranging from 27-54 g/100 g and the only silkworm was presented of 40% essential amino acids[14]. A few reports of using edible insects as a nitrogen source for *C. militaris* mycelium growth. This research aimed to evaluate the effect of edible insects on the *C. militaris* mycelium formation. This report will present the edible insect which is an alternative nitrogen source for *C. militaris* cultivation.

MATERIALS AND METHODS

Study area: All the experiments were performed during October, 2019 to June, 2020 in Agricultural Laboratory, Department of Agricultural Technology and Environment, Faculty of Science and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima, Thailand and the Microbiology Laboratory, Department of Science and Technology, Faculty of Liberal Arts and Science, Roi Et Rajabhat University, Roi Et, Thailand.

***Cordyceps militaris* strain:** The *C. militaris* strain was obtained from Chiang Mai, Thailand. The *C. militaris* stock culture was maintained on a Potato Dextrose Agar (PDA) bottle and stored at 4°C.

Edible insect samples: Silkworm (*Bombyx mori* L.), Eri silkworm (*Samia ricini* D.), House cricket (*Acheta domesticus* L.), Field cricket (*Gryllus bimaculatus* De Geer), Mealworm (*Tenebrio molitor* L.), Sago palm weevil (*Rhynchophorus ferrugineus*) and Giant water bug (*Lethocerus indicus*) were purchased from local organic insect farms, Nakhon Ratchasima, Thailand.

Chemicals and reagents: Potato Dextrose Agar (PDA) and Bacterial Agar were purchased from HiMedia (HiMedia Laboratories Pvt. Ltd, India).

Nitrogen and protein content analysis: Edible insects were dried at 60°C until completely dried. The Nitrogen and crude protein content were measured using the Kjeldahl method[15].

Effect of boiled and dried edible insects on the *Cordyceps militaris* mycelium formation: The boiled edible insects were blended and filtered. The filtrated liquid was mixed with Potato Dextrose Agar (PDA) at the ratio of 1:10 (w/v) (Table 1). The dried edible insects were dried at 50°C and blended into fine powder. The edible insect powder was mixed with Potato Dextrose Agar (PDA) at the ratio of 1:10 (w/v) (Table 1). The mixer of edible insect and PDA was sterilized at 121°C for 20 min before poured into a sterile Petri dish. The 0.5 cm diameter of PDA cultured *C. militaris* for 14 days was transferred into the centre on PDA mixed with each boiled edible insect. The PDA plates were incubated in the dark at 22°C for 21 days. The experimental design was used the

Table 1: *Cordyceps militaris* culture medium composition

Method	Culture medium
1	PDA (control)
2	PDA+silkworm (*Bombyx mori* L.)
3	PDA+Eri silkworm (*Samia ricini* D.)
4	PDA+house cricket (*Acheta domesticus* L.)
5	PDA+field cricket (*Gryllus bimaculatus* de geer)
6	PDA+sago palm weevil (*Rhynchophorus ferrugineus*)
7	PDA+mealworm (*Tenebrio molitor* L.)
8	PDA+giant water bug (*Lethocerus indicus*)

PDA: Potato Dextrose Agar

Completely Randomized Design (CRD) consisting of 8 methods, 3 replications per method and 5 dishes per replication. The growth of *C. militaris* was determined using the diameter of the fungal colony on day 7, 14 and 21.

Data analysis: The data for each experiment were expressed as mean±standard error. The analysis of the variance and comparison of the differences in the mean diameter of each method was calculated using Duncan's New Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Nitrogen and protein content: The seven types of edible insects purchased from local organic insect farms, Thailand. All edible insect was dried and measured of nitrogen and protein content using Kjeldahl method. The results presented that the highest of nitrogen and protein content were at 12.01 and 75.04% was found in Eri silkworm (*Samia ricini* D.) and followed by at 10.46 and 65.36% from House cricket (*Acheta domesticus* L.) and the lowest of nitrogen and protein content were presented in Sago palm weevil (*Rhynchophorus ferrugineus*) (3.92 and 24.52%) (Table 2). The protein content of Eri silkworm (*Samia ricini* D.) was higher than Cockroaches (*Blattodea*), Beetles (*Coleoptera*), Flies (*Diptera*), Beetles (Hemiptera), Bees, wasps, ants (*Hymenoptera*), Termites (*Isoptera*), Caterpillars (Lepidoptera), Dragonflies (*Odonata*), Grasshoppers, locusts, crickets (*Orthoptera*) which reported by Rumpold, B.A. and O. Schlüter[16]. The protein content of Sago palm weevil (*R. ferrugineus*) was lower than previously reported by Kim *et al.*[17] that presented the protein content of *Rhynchophoru sphoenicis* as 28-50%.

Effect of nitrogen source from boiled edible insects on *C. militaris* mycelium growth: The boiled edible insects were used as nitrogen source and were mixed with PDA at the ratio of 1:10 (w/v). The growth of *C. militaris* on each type of edible insects was determined using the diameter of the fungal colony on day 7, 14 and 21. The results of nitrogen source from a boiled edible insect at day 7 indicated that the *C. militaris* cultured on PDA (control) had the highest colony diameter (29.00 mm) but was not significantly different from cultured on PDA+Silkworm (*Bombyx mori* L.) (28.67 mm). The smallest colony diameter was found when cultured using PDA+Mealworm (*Tenebrio molitor* L.) (23.91 mm). The results of nitrogen source from a boiled edible insect at day 14 presented that the *C. militaris* cultured on PDA+Silkworm (*Bombyx mori* L.) had the highest diameter (64.48 mm) but were not statistically different from those cultured on PDA (63.83 mm) and PDA+Eri silkworm (*Samia ricini* D.) (60.16 mm). The smallest colony diameter was found when cultured using PDA+House cricket (*Acheta domesticus* L.) (52.67 mm). The results of nitrogen source from a boiled edible insect at day 21 indicated that the highest colony diameter was found when cultured with PDA+Silkworm (*Bombyx mori* L.) (88.00 mm), followed by PDA+Giant water bug (*Lethocerus indicus*) (85.50 mm), PDA+Sago palm weevil (*Rhynchophorus ferrugineus*) (85.33 mm), PDA (84.33 mm) and PDA Eri silkworm (*Samia ricini* D.) (83.67 mm), respectively (Table 3). The growth of *C. militaris* mycelium has a white colony and grow smooth with the surface of the PDA Fig. 1(a-h).

Effect of nitrogen source from dried edible insects on *C. militaris* mycelium growth: The dried edible insects were used as nitrogen source and were mixed with PDA at the ratio of 1:10 (w/v). The growth of *C. militaris* mycelium on each type of edible insects was determined using the diameter of the fungal colony on day 7, 14 and 21. The results of day 7 indicated that the largest diameter at 29.50 mm was obtained when cultured with only PDA and significantly different (p<0.01) from PDA mixed with dried insect. The smallest diameter was found at 18.33 mm when cultured using PDA+ Silkworm (*Bombyx mori* L.). The results of day 14 and 21 demonstrated that the highest diameter at 63.83 and

Table 2: Nitrogen and protein content in edible insects

	Percentage of dry matter#	
Edible insect	Nitrogen	Protein
Eri silkworm (*Samia ricini* D.)	12.01±0.09A	75.04±0.57A
Silkworm (*Bombyx mori* L.)	8.39±0.37D	52.44±2.96D
Giant water bug (*Lethocerus indicus*)	7.97±0.07E	49.79±0.45E
Field cricket (*Gryllus bimaculatus* de geer)	8.94±0.08C	55.87±0.52C
House cricket (*Acheta domesticus* L.)	10.46±0.06B	65.36±0.39B
Mealworm (*Tenebrio molitor* L.)	7.85±0.04E	49.06±0.29E
Sago palm weevil (*Rhynchophorus ferrugineus*)	3.92±0.17F	24.52±1.10F
p-value	<0.0001	<0.0001
CV (%)	1.95	1.95

#Means having the same letter(s) are not significantly different by DMRT (p>0.05), and CV: Coefficient of variation

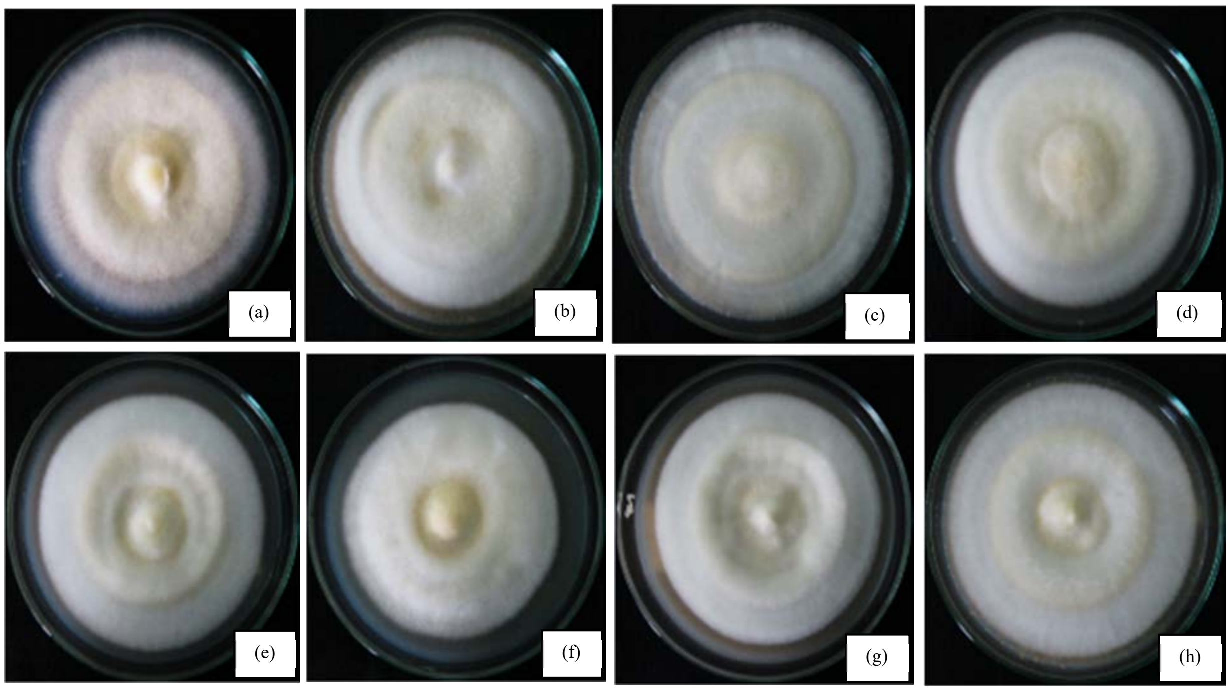

Fig. 1(a-h): *Cordyceps militaris* mycelium growth on PDA containing boiled edible insects
(a) PDA (control), (b) PDA+Eri silkworm, (c) PDA+silkworm, (d) PDA+giant water bug, (e) PDA+field cricket, (f) PDA+house cricket, (g) PDA+mealworm and (h) PDA+sago palm weevil

Table 3: *Cordyceps militaris* colony diameter cultured on PDA mixed with boiled edible insects

	C. militaris colony diameter (mm)#		
Culture medium	Day 7	Day 14	Day 21
PDA (control)	29.00±0.89[A]	63.83±1.26[A]	84.33±3.01[AB]
PDA+Eri silkworm	25.41±1.42[BC]	60.16±2.56[AB]	83.67±4.72[ABC]
PDA+silkworm	28.67±0.75[A]	64.48±3.26[A]	88.00±0.87[A]
PDA+giant water bug	26.33±0.51[B]	55.00±3.97[CD]	85.50±1.00[AB]
PDA+field cricket	25.75±2.07[B]	53.33±2.08[D]	80.00±1.73[BC]
PDA+house cricket	25.25±1.21[BC]	52.67±2.08[D]	77.50±5.89[C]
PDA+mealworm	23.91±1.24[C]	54.50±2.00[CD]	79.00±4.58[BC]
PDA+sago palm weevil	25.41±0.97[BC]	58.67±1.15[BC]	85.33±0.29[AB]
p-value	<0.0001	<0.0001	<0.0178
CV (%)	4.65	4.26	4.09

#Means having the same letter(s) are not significantly different by DMRT (p>0.05). Potato Dextrose Agar (PDA) and CV: Coefficient of variation

84.33 mm was obtained from cultured using PDA+House cricket (*Acheta domesticus* L.) and the smallest diameter at39.83 mm and 62.33 mm was presented when growing with PDA+Field cricket (*Gryllus bimaculatus* De Geer) (Table 4). The growth of *C. militaris* mycelium has a white colony and grow smooth with the surface of the PDA Fig. 2(a-h).

Effect of nitrogen source from dried edible insects on *C. militaris* mycelium growth: The dried edible insects were used as nitrogen source and were mixed with PDA at the ratio of 1:10 (w/v). The growth of *C. militaris* mycelium on each type of edible insects was determined using the diameter of the fungal colony on day 7, 14 and 21. The results of day 7 indicated that the largest diameter at 29.50 mm was obtained when cultured with only PDA and significantly different from PDA mixed with dried insect. The smallest diameter was found at 18.33 mm when cultured using PDA+Silkworm (*Bombyx mori* L.). The results of day 14 and 21 demonstrated that the highest diameter at 63.83 and 84.33 mm was obtained from cultured using PDA+House cricket (*Acheta domesticus* L.) and the smallest diameter at 39.83 and 62.33 mm was presented when growing with PDA+Field cricket (*Gryllus*

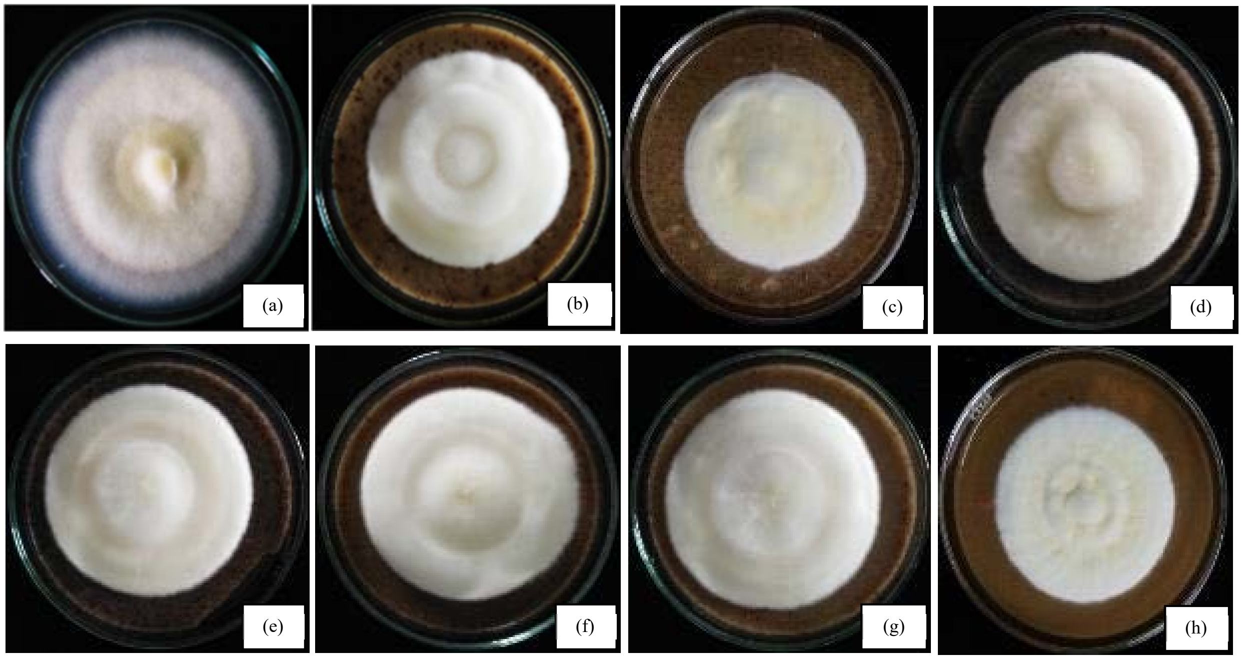

Fig. 2 (a-h): *Cordyceps militaris* mycelium growth on PDA containing dried edible insects
(a) PDA (control), (b) PDA+Eri silkworm, (c) PDA+silkworm, (d) PDA+giant water bug, (e) PDA+field cricket, (f) PDA+house cricket, (g) PDA+mealworm and (h) PDA+sago palm weevil

Table 4: *Cordyceps militaris* colony diameter cultured on PDA mixed with dried edible insects

	C. militaris colony diameter (mm)#		
Culture medium	Day 7	Day 14	Day 21
PDA (control)	29.50±1.00^{A}	47.67±2.57^{B}	71.50±5.68^{B}
PDA+Eri silkworm	21.83±1.53^{BC}	41.83±3.69^{CD}	69.00±4.09^{B}
PDA+silkworm	18.33±0.58^{D}	47.17±1.60^{B}	70.50±2.29^{B}
PDA+giant water bug	23.50±0.50^{B}	45.67±0.58^{BC}	71.33±1.60^{B}
PDA+field cricket	22.67±1.26^{BC}	39.83±2.47^{D}	62.33±5.06^{C}
PDA+house cricket	21.00±1.00^{C}	63.83±1.26^{A}	84.33±3.01^{A}
PDA+mealworm	23.17±1.04^{BC}	48.83±1.04^{B}	74.33±1.26^{B}
PDA+sago palm weevil	18.67±2.47^{D}	44.67±3.21^{BC}	72.00±1.32^{B}
p-value	<0.0001	<0.0001	0.0001
CV (%)	5.85	4.84	4.79

#Means having the same letter(s) are not significantly different by DMRT (p>0.05). Potato Dextrose Agar (PDA) and CV: Coefficient of variation

bimaculatus De Geer) (Table 4). The growth of *C. militaris* mycelium has a white colony and grow smooth with the surface of the PDA Fig. 2(a-h).

The result from this research was according to Kang *et al.*[18] reported the excess of nitrogen was promoted a faster mycelial growth and the organic nitrogen was advantageous to both the growth and biosynthesis of metabolites. Sari *et al.*[19] were reported the effect of 10% Cricket (*Gryllus testaceus*) powder supplemented in SDAY can increase the mycelial growth of the insect-pathogenic fungus *Metarhizium majus* UICC 295. Sunita *et al.*[20] was presented about the *M. anisopliae* showed the highest of mycelium growth and sporulation when cultured using SDA+nymph/adult of *Pyrilla*. Kwon *et al.*[21] reported that the highest mycelial growth at 11.93 g L^{-1} was obtained when the organic nitrogen (yeast extract) was used. *Lui et al.*[22] reported that organic nitrogen source including yeast powder, peptone and the beef extract was suitable for *Isaria farinosa* mycelium growth. This research presented that the high nitrogen source might not major effected mycelium growth but other compounds including fats, carbohydrates, minerals, amino acids and secondary metabolites such as flavonoids, alkaloids, polysaccharides, hormones and phospholipids which content in edible insects will be affected[23]. This research can be applied for edible insect powder production used as an organic nitrogen source for *C. militaris* cultivation and increased the value-added of edible insects.

CONCLUSION

The results of nitrogen source from a boiled edible insect at day 21 indicated that the highest colony diameter at 88.00 mm was obtained when cultured with PDA+*B. mori* L. The results of nitrogen source from a dried edible insect at day 21 presented that the highest diameter at 84.33 mm was obtained from cultured using PDA+*A. domesticus* L. The culture media containing edible insects is a good alternative way that can be increased the growth of *C. militaris* mycelium which is the important stage for *C. militaris* cultivation.

SIGNIFICANCE STATEMENT

This study discovers the effect of nitrogen sources from edible insects on the growth of *C. militaris* mycelium that can be beneficial for *C. militaris* cultivation. This study will help the researcher to uncover the critical areas of using edible insects as a nitrogen source for *C. militaris* cultivation that many researchers were not able to explore. Thus, a new application using the nitrogen sources from edible insects on the growth of *C. militaris* may be arrived at.

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